Diarrhea is an important cause of morbidity and mortality in all regions of the world and among all ages. In developing countries, approximately two million deaths are recorded per year in the children under-five years of age group alone (Kosek et al., 2003). The term “Diarrhea” in Greek means, “to flow through like a stream”. It is the condition of having three or more loose or liquid bowel movements per day. By definition, acute diarrhea continues for ≤14 days; persistent diarrhea, for more than 14 days; and chronic diarrhea, for 30 days (Carey et al., 2008). Recent research in Brazil has shown that access to improved sanitation alone reduced the rate of childhood diarrhea by up to 43% and hand washing with soap at critical times alone has been shown to reduce the incidence of diarrhea by 45% (Cumming, 2009). In Indian perspective the sanitation conditions have to be improved substantially to control the widespread diarrheal disease throughout the nation (Agoramoorthy, 2009). Diarrhea outbreaks are not still uncommon in India (Das et al., 2009). Planning and evaluation of interventions to control diarrhea deaths and to reduce under-5 mortality is obstructed by the lack of a system that regularly generates cause-of-death information. The methods used widely provide country-level estimates that constitute alternative information for planning in settings without adequate data (Boschi-Pinto et al., 2008). Many children in low- and middle-income countries including India do not receive proper treatment for diarrhea points to the urgency in addressing this unfinished agenda in child survival. The effectiveness of diarrhea control needs to be improved after critical review of established approaches and activities to reach caregivers of children at risk of dying from diarrheal diseases. Significant efforts must be made to scale up activities to improve case management and reduce childhood deaths from diarrhea (Forsberg et al., 2007).
Research to address the etiology of diarrhea should be of much interest as the knowledge of etiology directly affects management and strategy planning for prevention of a disease. Diarrhea caused by *Yersinia enterocolitica* is now a global concern and it is recommended that during autumn-winter months, clinicians should pay more attention to clinical manifestations, early diagnosis, and treatment with susceptible antibiotics of yersiniosis and its complications, targeting high-risk patients (Zheng *et al*., 2008).

The genus *Yersinia* is named after Alexander Yersin, a Swiss bacteriologist who isolated the plague bacillus. He named the bacteria *Pasteurella pestis* due to its resemblance to other members of the same genus (Butler, 1994). Although earlier the bacterium was termed plague bacillus (now renamed as *Yersinia pestis*), once the taxonomy of the genus *Yersinia* was unraveled, it was found that the first species to have been described was in fact *Y. pseudotuberculosis*. Malassez and Vignal described a disease in guinea pigs in 1883 characterized by nodules in the internal organs resembling tuberculosis (Schiemann, 1989). The causal organism was named *Bacillus pseudotuberculosis rodentium* by Pfeiffer in 1889 which later became *Pasteurella pseudotuberculosis* (Bottone, 1981). A year after Yersin’s death, the bacterium was placed in a new genus *Yersinia* in 1944 by Van Loghem (Van Loghem, 1944). This new genus was included in family Enterobacteriaceae as proposed by Frederiksen in 1964.


*Yersinia enterocolitica* is a pleomorphic gram-negative bacillus. As a human pathogen, *Y. enterocolitica* is most frequently associated with acute diarrhea, terminal ileitis, mesenteric lymphadenitis, and pseudoappendicitis. The bacterium was first isolated from a facial abscess of a 53-year-old farmer in USA.
in 1934. It was named *Flavobacterium pseudomalle* (Bottone, 1981). Schleifstein and Coleman (1939) provided the first recognized description of 5 human isolates and proposed the name *Bacterium enterocoliticum*. Over the next three decades, the bacterium was variously names *Pasteurella pseudotuberculosis X*, *Pasteurella pseudotuberculosis type b*, *Pasteurella X*, *Pasteurella pseudotuberculosis atypique* and *Les germes X* (Bottone, 1981). In 1973, Knapp and Thal established the similarity between these strains from America and Europe and in 1964, Frederiksen demonstrated that strains from his collection were distinct from *Y. pseudotuberculosis*, but were similar enough to be designated as a separate species within the genus, which he named *Yersinia enterocolitica*.

**Isolation of *Y. enterocolitica***

*Y. enterocolitica* is isolated commonly from the faecal specimens of patients. The use of enrichment broths such as selenite F has been found to improve the recovery of the organism from faecal specimens (Ratnam et al., 1982). The bacterium grows well on standard plate media such as MacConkey agar, although its growth is slower than other Enterobacteriaceae, colonies are easily overlooked when cultivated at 37 °C. The performance of media used for the recovery of *Y. enterocolitica* is therefore improved by incubation at lower temperatures, e.g. 22-28 °C, for 48 h. A number of selective media designed for the isolation of enteric bacteria have been evaluated for the recovery of *Yersinia* (Head et al., 1982; Harmon et al., 1983), however, a specialized agar medium, CIN agar, synthesized by Schiemann in 1979, has proved to be the most effective plating medium for these organisms (Schiemann, 1979).

The isolation of *Y. enterocolitica* from asymptomatic people, animal reservoirs, foods or environment has proved difficult, due to the presence of small numbers of organisms and their rapid overgrowth by other competitive bacteria. Thus, the recovery of these bacteria from such sources has been widely investigated and many enrichment protocols have been assessed (Schiemann, 1989). Cold-enrichment techniques, based on the ability of *Y. enterocolitica* to
multiply at refrigerated temperatures, have been found to be the most successful, and a 3-week enrichment at 4 °C in PBS (pH 7.6), either alone or supplemented with sorbitol, bile salts or mannitol, has been used for many years for the recovery of *Yersinia* from a range of samples (Ahvonen, 1970; Greenwood *et al.*, 1975; Pai *et al.*, 1979). Despite its widespread usage, cold-enrichment in PBS is believed by some workers to preferentially enhance the isolation of environmental strains of *Yersinia* and other methods, such as post-enrichment alkali treatment (Aulisio *et al.*, 1980, Doyle and Hugdahl, 1983) and two step enrichments, have also been devised and evaluated for the preferential recovery of pathogenic strains of *Y. enterocolitica* from mixed cultures. Van Pee and Stragier (1979), evaluated several cold-enrichment media and showed that the enrichment quotient achieved after 3 weeks at 4 °C was highly dependent on the initial cell concentration and the medium used. Their findings indicated that media for cold-enrichment should be of high nutritional value as enrichment in tryptone-soya broth yielded better results than the frequently used PBS. Wauters *et al.* (1988a) used a new enrichment medium, ITC, and found that it was more sensitive for the recovery of *Y. enterocolitica* O:3 strains than either cold-or two-step enrichments.

**Identification of *Y. enterocolitica***

Suspect colonies of agar plates are further characterized by inoculating triple sugar ion (TSI), two semisolid tubes of motility media and a urea agar slant. All media are incubated at 25 °C except one of the motility media, which is incubated at 37 °C. The TSI reaction at 24 hrs is an acid slant, acid butt, with no gas or H₂S. *Y. enterocolitica* is non-motile at 37 °C, motile at 25 °C and urea positive (Morris and Feeley, 1976). The identity of *Y. enterocolitica* can be confirmed by the following biochemical tests (incubated at 25 °C): sucrose, rhamnose, raffinose, melibiose, alpha-methylglucoside and Simmon’s citrate. *Y. enterocolitica* is sucrose-positive and negative in the other tests (Schiemann, 1989). Sucrose-negative strains of the organism have been isolated in Japan (Fukushima *et al.*, 1988). The complete biochemical characterization of *Y. enterocolitica sensu strict* was described by Bercovier *et al.* (1980a). The API 20
E mictrotube biochemical system has been used for the identification of *Y. enterocolitica* with excellent results, providing incubation as performed at 28 °C rather than at 37 °C as recommended by the manufacturer (Sharma et al., 1990).

**Biotyping of *Y. enterocolitica***

Biotyping has been extensively used because *Y. enterocolitica* comprises a biochemically heterogenous group of bacteria (Bercovier et al., 1980a). The biotyping scheme was first proposed by Nilehn (1973). It was based on the following reactions; lactose oxidation, indole production, acid from sucrose, sorbitol, trehalose and xylose, nitrate reduction, beta galactosidase (ONPG) activity, Voges-Proskauer reaction, and esculin hydrolysis. Although the criteria for inclusion in a biotype have been amended by successive workers (Bercovier et al., 1980a; Wuters et al., 1987), this method for subtyping the species is widely used (Fukushima et al., 1984b). *Y. enterocolitica* has been divided into six different biotypes: IA, IB, 2-5. Biotype IA consists of non-pathogenic strains, and biotype IB and 2-5 include strains that are associated with disease in man and animals. The most widespread strains of *Y. enterocolitica* belong to biotype 4. (Cimboi et al., 1994; Burnens et al., 1996; Wauters et al., 1987).

**Serotyping**

Strains of *Y. enterocolitica* can also be phenotyped on the basis of serotypes. Serotyping is mostly based on UPS surface O antigen, and more seldom on H (flageller) or K (fimbriae) antigens. Since the initial description of Winblad (1967) of eight O antigens, the list has been extended to 76. Wauters et al. (1991) have proposed a revised and simplified typing scheme, which includes 20 antigenic factors for *Y. enterocolitica* alone. Serotype 0:3 is most frequently isolated from humans globally. Other serotypes obtained from humans include serotype 0:9 and 0:5,27, particularly in Europe, and serotype 0:8 in the USA. However, several O antigens, including 0:3, 0:8 and 0:9, have been found in both pathogenic and non-pathogenic strains (Aleksic, 1995). An accurate biochemical characterization is needed before or after serological typing to allow for correct
assessment of the relevance of strains especially from foods and the environment, since related species and biotype IA strains are widely distributed in these samples (Wauters et al., 1991; Hoorfar and Holmvig, 1999).

**Phage typing**

Two schemes (Swedish and French) are used for phage typing of *Y. enterocolitica* (Schiemann, 1989). Of these, the French scheme has been used more often and recognises 12 phage types: I-X (including IXa-c). The Swedish scheme recognises seven phages (A1, A2, B1, B2, C32, C61, E1) and is used less frequently. Neither of these schemes has produced a large number of distinct epidemiological types because many strains fall into the same phage types. Strains of bioserotype 4/0:3 and phage type VIII predominate in Europe and Japan (Kapperud, 1991), whereas phage type IXb has been isolated in Canada (Toma and Deidrick, 1975) and in the USA (Doyle et al., 1981). Baker and Farmer III (1982) have developed a set of 24 phages, which offers a marked improvement for differentiation. Because of the need to maintain stocks of biologically active phages and control strains, phage typing is available at only a few laboratories (Pulkkinen et al., 1986; Nesbakken et al., 1987; Kwaga and Iversen, 1993).

**Restriction endonuclease analysis of the plasmid (REAP)**

Plasmid analysis, the first bacterial typing tool, has been used for differentiating bacterial strains (Farber, 1996). Plasmids are isolated from each isolate and then separated electrophoretically in an agarose gel to determine their number and size. Pathogenic strains of *Y. enterocolitica* contain only one virulence plasmid of about 70 kb (Vesikari et al., 1981; Heesemann et al., 1983; Skurnik et al., 1983). To increase discriminatory power, the isolated plasmid is cut with different frequent-cutting restriction enzymes. Restriction endonuclease analysis of the plasmid (REAP) yields specific patterns for each bioserotype. However, within bioserotype 4/0:3, the diversity of the REAP patterns is limited. (Heesemann et al., 1983; Nesbakken et al., 1987; Kwaga and Iversen, 1993)
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Restriction endonuclease analysis of the chromosome (REAC)

Chromosomal DNA restriction analysis was the first of the chromosomal DNA-based typing schemes. In this method, endonucleases with relatively frequent restriction sites are used to cut the DNA, thereby generating hundreds of fragments ranging from 0.5 to 50 kb in size (Maslow et al., 1993). A major limitation of this technique is the difficulty in interpreting complex profiles, which consist of hundreds of bands that may be unresolved and overlapping. Kapperud et al. (1990) have used REAC to study polymorphism in restriction fragment patterns among Y. enterocolitica isolates belonging to different bioserotypes. A total of 22 distinct REAC patterns were distinguished among the 72 Yersinia strains examined, and the patterns varied clearly between bioserotypes.

Ribotyping

To avoid problems associated with complex REAC patterns, probes, which hybridise to specific DNA sequences, are used. Ribotyping refers to the use of nucleic acid probes to recognize ribosomal genes, which are present in all bacteria (Farber, 1996). Chromosomal DNA is isolated and a frequent-cutting enzyme is used to cut the DNA into small fragments. Fragments are separated by electrophoresis through an agarose gel. The separated DNA fragments are transferred from the agarose to either a nitrocellulose or nylon membrane by Southern blotting (Southern, 1975). Probing is usually done with labelled probes containing E. coli 23S, 16S and 5S rRNA sequences. After probing, fragments containing a ribosomal gene are highlighted, creating a fingerprint pattern containing approximately 1 to 15 bands that can be compared easily among isolates (Anderson and Saunders, 1990; Blumberg et al., 1991; Mendoza et al., 1996).

Ribotyping has been used to characterise Y. enterocolitica isolates in several studies (Andersen and Saunders 1990; Blumberg et al., 1991; Mendoza et al., 1996; Lobato et al., 1998; Fukushima et al., 1998). A close relationship has been found between the ribotypes and bioserotypes of Y. enterocolitica isolates. Although variation between ribotypes exists among isolates belonging to the
same bioserotype, genetic diversity is limited among isolate of bioserotype 4/0:3. Pulse Field Gel Electrophoresis (PFGE) is a variation of agarose gel electrophoresis that permits analysis of large fragments of bacterial DNA. For PFGE, bacterial isolates grown either in broth or on solid media are combined with molten agarose and poured into small moulds. The embedded bacteria are then subjected to in situ detergent-enzyme lysis and digestion with an infrequently cutting restriction enzyme. The digested bacterial plugs, containing the whole genome, are inserted into an agarose gel and subjected to electrophoresis in an apparatus in which the polarity of the current is changed at predetermined intervals. The pulse field allows clear separation of large molecular length DNA fragments, ranging from 10 kb to 800 kb. PFGE provides a highly reproducible restriction profile, which typically shows distinct, well-resolved fragments representing the entire bacterial genome in a single gel (Logonne, 1993). Because of the high discriminatory power, and good intra- and interlaboratory reproducibility, PFGE is still one of the best methods available when compared with the newer typing methods (Olive and Bean, 1999).

A number of studies have been conducted to characterise *Y. enterocolitica* with PFGE (Iteman et al., 1991; Buchrieser et al., 1994; Najdenski et al., 1994; Saken et al., 1994; Hosaka et al., 1997). PFGE allows subtyping of strains belonging to same bioserotype (Buchrieser et al., 1994; Najdenski et al., 1994; Saken et al., 1994). Najdenski et al (1994) showed that the pulsotype resembles the biotype more closely than the serotype and that the genome of *Y. enterocolitica* is stable in vitro. The global homogeneity of the pulsotypes among strains of bioserotype 40:3 has been shown to be high. Although strains of bioserotype 4/0:3 can be subdivided into several pulsotypes, most strains fall into one or two dominating pulsotypes, decreasing the discriminatory power of PFGE. (Najdenski et al., 1994; Saken et al., 1994; Asplund et al., 1998).

**Randomly amplified polymorphic DNA (RAPD)**

Randomly amplified polymorphic DNA (RAPD) assay, also referred to as arbitrary primed PCR; is a variation of the PCR technique employing a single
short (typically 10 base pairs) primer that is not targeted to amplify any specific bacterial sequence. The primer hybridises at multiple random chromosomal locations and initiates DNA synthesis at low annealing temperatures. The resulting PCR products present a variety of different-sized DNA fragments that are visualized by agarose gel electrophoresis (Farber, 1996). RAPD is a very simple and quick method, but its reproducibility is low and standarisation of the technique is difficult (Olive and Bean 1999). Some studies have characterized *Y. enterocolitica* isolates with RAPD (Rasmussen et al., 1994; Odinot et al., 1995; Leal et al., 1999). The method allows discrimination between *Y. enterocolitica* isolates belonging to different bioserotypes and, also in some cases, between isolates belonging to the same bioserotype (Odinot et al., 1995; Leal et al., 1999).

### Animal Reservoirs

#### Pigs

The earliest isolation of *Y. enterocolitica* from healthy pigs was by Dickinson and Mocquotin 1961, in a study of the bacterial flora of the alimentary tract (Dickinson and Mocquot, 1961). Although they called the organism they had isolated *Pasteurella pseudotuberculosis* type b, examination of the biochemical reactions of the 16 strains recovered shows that they were indole, xylose, salicin and esculin negative, and thus almost certainly belong to biotype 4. Since then, slaughter house surveys carried out in Europe (Christensen, 1980; Nesbakken and Kapperud, 1985; de Boer and Nouws, 1991), North America (Toma and Deidrick, 1975; Hariharan et al., 1995) and Japan (Fukushima et al., 1990; Shiozawa et al., 1991) have shown that pathogenic strains of *Y. enterocolitica* are common commensals of pigs worldwide, although regional differences in bioserotype distribution are recorded. Despite the fact that human infections with *Y. enterocolitica* are reported mainly from temperate regions, pigs in sub-tropical and tropical regions also appear to carry the organism, possibly because many original populations were established with breeding stock from Europe and Canada (Chen et al., 1983). While early surveys of pigs attempted to isolate the
organism from faeces and intestinal contents (Hurvell, 1981; Christensen, 1980), later studies showed that the frequency of isolation of serotype 0:3 from the tongue and palatine tonsils of pigs at slaughter was approximately ten times greater than that reported for other sites, with isolation rates of up to 56% being recorded (Pedersen, 1979; Wauters, 1979; Schiemann, 1980; de Boer et al., 1986). The oral cavity of pigs is believed to be the natural reservoir of human pathogenic strains of \textit{Y. enterocolitica} and removal of the head before processing, or excision of the tissues of the pharyngeal region, are recommended measures to reduce carcass contamination by the organism (Kapperud, 1991). Nevertheless, as the organism is also frequently isolated from faeces, a slaughtering procedure which prevents dissemination of yersiniae from the intestine to the carcass has also been recommended for the hygienic production of pork (Nesbakken and Borch, 1995). In addition to abattoir surveys, epidemiological studies have also focused on pig farms. In 1980, Christensen surveyed a number of Danish farms and found that although \textit{Y. enterocolitica} 0:3 had a farm-wide distribution, with herds either constantly positive or negative during a one-year period, the prevalence varied widely between herds. Furthermore, his farm survey revealed that the \textit{Yersinia}-positive farms were all of the open management type, where 6-8 week-old pigs were purchased from various sources, indicating that this was an important factor in the spread of the organism. In 1991, Andersen et al. further investigated the epidemiology of \textit{Y. enterocolitica} 0:3 in 99 Danish pig herds and found that 82\% of herds were infected, with an overall prevalence of 25\%. The 18\% of herds which were negative for \textit{Y. enterocolitica} were mostly represented by a limited number of pigs, however, no other herd-management factors were statistically associated with the presence of the organism. Fukushima et al. (1984a) examined pigs from 5 Japanese farms for their \textit{Y. enterocolitica} carrier status and found differences in the seasonal isolation rates between them. In heavily infected herds, \textit{Y. enterocolitica} was recovered in all months, whereas in other farms the organism was not recovered in summer. In addition, yersiniae were not recovered from
breeding animals, only from the younger fattening stock. They also investigated the transmission of *Y. enterocolitica* on the farms and found that piglets were infected within 2-7 weeks of being moved to a pen which had been washed thoroughly after previously housing another batch of infected animals. These findings led them to suggest that piglets were probably infected with a relatively small number of organisms and that infected faeces and pen floors were the most important source of infection. In a further experimental study, Fukushima *et al.* (1984a) demonstrated that pigs infected with either serotype 0:3 or 0:5,27 were protected from challenge by the heterologous strain, indicating that competitive exclusion might result in farms being only infected with a single strain. Serological surveys have been used to determine the *Y. enterocolitica* status of pig herds in Germany, UK and China (Chen *et al.*, 1983), and could be useful for the initial screening of farms prior to cultural studies with their inherent sensitivity problems. Surveys investigating the prevalence of *Y. enterocolitica* in slaughtered pigs and pig products have been hampered by overgrowth of the organism with other Enterobacteriaceae at 37°C incubation temperatures, and many enrichment methods have been used in an attempt to overcome this problem (Schiemann, 1979; Van Pee and Stragier, 1979; Head *et al.*, 1982; Wauters *et al.*, 1988a; deBoer, 1992). Most of these methods, however, make use of the ability of *Y. enterocolitica* to outgrow competitive microflora at 4°C, in a technique known as cold-enrichment. Cold-enrichment was first used in 1963 by Paterson and Cook for the recovery of *Pasteurella (Yersinia) pseudotuberculosis* from animal faeces and it is still widely used today. In its simplest form it involves incubation of a sample in phosphate buffered saline (PBS), pH 7.6, for three weeks, before plating onto solid media, although numerous combinations of selective and enrichment agents have since been included to achieve maximum isolation of pathogenic serotypes of the organism in a shorter period of time (Van Pee and Stragier, 1979; Wauters *et al.*, 1988a; Schiemann, 1989). A two-step enrichment procedure was shown by Nesbakken *et al.* (1985), to be superior to other methods for the recovery of pathogenic *Y. enterocolitica* serotypes from
pigs and was the method of choice for this study. The ability of *Y. enterocolitica* to survive and multiply at 4°C, the temperature used for preservation of chilled food, makes it of concern to the food industry (Hurvell, 1981; Kapperud, 1991) and pathogenic strains of the organism, probably arising from carcass contamination at the time of slaughter, have been recovered from meats, particularly pork and pork products (Schiemann, 1980; Andersen, 1988; Fukushima *et al.*, 1990). In addition, Danish study in 1987 recovered bioserotype 4/0:3 from the environment of 10% of pork butcher's shops, with a higher rate of contamination seen in small family butchers than in larger establishments (Christensen, 1987a). Nevertheless, despite the development of new enrichment media and protocols, and the frequent recovery of human pathogenic *yersinia* from freshly slaughtered pigs and pig carcasses, isolation from retail pork products has often been paradoxically low (Schiemann, 1980; Kapperud, 1991; de Boer, 1992). Nesbakken *et al.* (1991a), however, used a DNA hybridisation assay to detect *Y. enterocolitica* in naturally contaminated pork products and found that 60% contained virulent strains of the organism. Their results indicated that the occurrence of pathogenic *Y. enterocolitica* on Norwegian pork products was substantially higher than had been previously demonstrated by Nesbakken *et al.* (1985), and that pork represented an important potential source of human infection in Norway. Despite strong epidemiological evidence for the connection between *Y. enterocolitica* infections in pigs and human yersiniosis (Tauxe *et al.*, 1987; Ostroff *et al.*, 1994), few cases have been reported where pigs or pork products were directly implicated as the source of infection (de Boer and Nouws, 1991), although circumstantial evidence exists for their involvement. In 1976, Sebes *et al.* reported on a case of *Y. enterocolitica* in a 65-year-old pig farmer, suggesting that his occupation may have resulted in his infection. In 1980, Aulisio *et al.* described a large outbreak of *Y. enterocolitica* 0:13 involving consumption of infected pasteurised milk. On investigation, it was found that unsold milk was taken to a pig farm for feeding to the pigs and the milk crates stored near pig pens were shown to be contaminated with the same unusual serotype that was
recovered from human patients indicating a possible porcine source for the organism. Walker and Gilmour (1986), demonstrated that water runoff from a pig farm contained the same serotype of \textit{Y. enterocolitica} that had been isolated from the pigs, and suggested that pigs could infect people indirectly via contaminated water. In 1987, a large epidemiological study was carried out by Tauxe \textit{et al.} to determine the risk factors for human yersiniosis in Belgium. They found that \textit{Y. enterocolitica} infection was strongly associated with the consumption of raw pork, a delicacy in Belgium, and that the incidence of infection was lowest among non-pork eating groups, including muslims. More evidence for the role of pigs in human yersiniosis came in 1988 from a Spanish study of antibiotic resistance patterns in \textit{Y. enterocolitica} (Trallero \textit{et al.}, 1988). Unusual resistance was demonstrated to chloramphenicol and streptomycin-sulphonamide in a cluster of human isolates and in strains recovered from pigs and pork products during the same period, supporting the hypothesis that in Spain pigs form the reservoir and source of many human \textit{Y. enterocolitica} infections. In 1990, Joseph \textit{et al.} reported details of a large outbreak of \textit{Y. enterocolitica} infection that had occurred in a UK boarding school. A cohort study of the school population showed an independent association between gastrointestinal illness and contact with pigs on a small farm run by the pupils. Also in 1990, Lee \textit{et al.} described another large outbreak of yersiniosis among infants in Atlanta, Georgia. The illnesses were strongly associated with the household preparation of chitterlings, which are the large intestines of pigs. Although none of the infants had direct contact with the raw chitterlings, in nearly all cases the persons cleaning the pig intestines were also caring for the infants. In 1991, Ichinohe \textit{et al.} reported the first case of \textit{Y. enterocolitica} bioserotype1B/0:8 from Japan, in which the source was believed to be raw pork, although this was not confirmed. In 1994, a Norwegian case-control study to identify sources of infection for sporadic yersiniosis showed that people with \textit{Y. enterocolitica} infection reported having eaten significantly more pork items in the two weeks prior to their illness than controls (Ostroff \textit{et al.}, 1994). Patients were
also more likely than controls to have stated a preference for eating raw or rare meat.

Evidence for the involvement of pigs as a source of infection for pork butchers and abattoir workers was gathered via a serological survey in Finland in 1991 (Merilahti-Palo et al., 1991). Antibodies against Y. enterocolitica 0:3 were observed more often in butchers handling pig throats and intestines than in the sera of healthy blood donors. In addition, 30-40% of the workers reported diarrhea or abdominal pain in the 6 months preceding the study, although the symptoms did not correlate with the occurrence of antibodies. In another serological survey, Nesbakken et al. (1991b), examined sera from Norwegian slaughter house employees, veterinarians and military recruits for antibodies to Y. enterocolitica 0:3. They found that the prevalence of antibodies was higher among workers involved in the slaughtering process than among those from other areas in the plants or office staff. However, a relatively high prevalence was also found in military recruits from urban centers and thus no final conclusions could be drawn regarding pig contact as a risk factor for yersiniosis. Robins-Browne et al. (1985) fed virulent serotype 0:3 strains to neonatal piglets and produced clinical illness, with intestinal lesions similar to those reported in people. The clinical response was dose related, ranging from subclinical or mild illness in animals given 2 x 10^9 cfu, to death in those given 4 x 10^10 cfu. In 1988, Schiemann studied the pathogenicity of Y. enterocolitica serotypes 0:3, 0:8, 0:21 and 0:13 in piglets by oral challenge of two litters, one caesarean derived and colostrum-deprived and the other born naturally. Of eight caesarean-derived piglets, four died or were destroyed because of severe illness, however, the other four, and all the naturally-born piglets, showed no clinical symptoms of disease. In 1995, Shu et al. orally infected newborn, colostrum-deprived piglets with 3 x 10^10 cfu of a human isolate of Y. enterocolitica 0:3 in an attempt to develop an animal model for yersiniosis. Of 14 infected piglets, 11 became anorexic, 5 vomited and 13 developed diarrhoea. Damage to the mucosa was observed in the whole gastrointestinal tract, but was more severe in the small intestine and
caecum. It was hypothesised that hypoacidity in the newborn stomach may have produced favourable conditions for bacterial invasion, and a similar situation may have occurred in the pigs in China, leading to symptoms of gastroenteritis.

**Dogs and cats**

In 1973, circumstantial evidence was published that implicated sick dogs as the source of infection in a large inter-familial outbreak of *Y. enterocolitica* enteritis involving sixteen people, two of whom died (Gutman et al., 1973). Five puppies from a litter of nine had died of a diarrheal illness the week before the first human case and the children of both families had cared for the puppies when they were ill. Unfortunately, surviving dogs were destroyed at the request of the families after the onset of the outbreak and without culture of their faeces.

In another case in the USA in 1976, involving one child, faecal specimens were examined from three surviving puppies in a litter belonging to the family, in which eight animals had died of a wasting illness the month before the child became ill (Wilson et al., 1976). *Yersinia enterocolitica* bioserotype 1B/O:20, was isolated from the animals, dried canine stool specimens and the sick child. No further reports of the involvement of dogs in human yersiniosis have been made.

Surveys carried out in Denmark and Japan have shown that dogs may carry strains of *Y. enterocolitica* potentially pathogenic for people, however, the prevalence in dogs was far lower than that commonly reported in pigs (Fukushima et al., 1984a; Andersen, 1988). Danish workers isolated *Y. enterocolitica* bioserotype 4/0: 3 from dogs on two occasions. Isolates were obtained from 1140 (2.5%) dogs examined in 1976 (Pedersen, 1979) and from 2/115 (1.7%) dogs examined in 1979. In Japan in 1977, Kaneko et al. isolated *Y. enterocolitica* serotypes 0:3, 0:5, 27 and 0:9 from 23/451 (5.1%) dogs. No other reports of the carriage of serotype 0:9 by dogs have been made. In 1978, Yanagawa et al. examined a further 704 dogs and recovered serotypes 0:3 and 0:5, 27 from 40 (5.7%). Fukushima et al. (1984b), also recovered these serotypes from a number of dogs, with puppies found to be most frequently infected with 0:3 and adults with 0:5, 27. In addition to these surveys, sporadic reports of the
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isolation of serotypes 0:3 and 0:5,27 from canine sources have been made from Italy (Tiscar et al., 1992), Spain (Ferrer et al., 1987), Brazil (Falcao, 1987) and England.

Other animals

Early investigators were interested primarily in sources of Y. pseudotuberculosis, as this organism was responsible not only for human infections, but also for disease in a wide range of animal species, including farm animals, domestic pets, wild mammals and birds (Schiemann, 1989). Despite a rising incidence of human infections in Europe and North America in the 1960's, interest in Y. enterocolitica in animals was initially stimulated by epizootics of bioserotype 3/0:1,2,3 infection in chinchillas in the USA, with the subsequent export of infected animals to Europe (Nilehn, 1973; Schiemann, 1989), and by hare mortalities in Europe caused by bioserotype 5/0:2,3 (Mair, 1973). The serotype 0:3 was recovered from a diarrheic cow in Brazil biotype 4 (0:3) from a bovine abortion in the UK (Brewer and Corbel, 1983), serotypes 0:3 and 0:9 from sheep in Russia, 0:9 from abortions in Indian buffaloes (Das et al., 1986), from raw goats' milk in the UK (Wale et al., 1991) and from healthy cattle and goats in France. Antibodies to serotype 0:3 were also found in various domestic animal species in Nigeria. In addition, serotype 0:5,27 (recognized as a human pathogen more frequently in North America and Japan than Europe) appeared to have an even wider host range than serotypes 0:3 and 0:9, including cattle (Brewer and Corbel, 1983), raccoon camel and chinchilla (Toma and Lafleur, 1974), sheep (Brewer and Corbel, 1983) and unspecified wild animals and birds (Shayegani et al., 1981). These results indicated that, while pigs and occasionally dogs and cats were probably the principal species harbouring serotypes potentially pathogenic for people, other animal species could well be involved in the epidemiology of human yersiniosis.

Several investigations suggested that water may act as a reservoir for Y. enterocolitica and infection due to bacterium may be transmitted by consumption of contaminated water. Y. enterocolitica has been isolated from water by number
of investigators (Fukushima, 1984a). Drinking water has been relatively widely investigated (Christensen, 1980; Stengel, 1986; Schiemann, 1990; Gonui and Karapinar, 1991) and in these studies, water has been shown to be a significant reservoir for non-pathogenic *Y. enterocolitica*. However, in epidemiological studies, untreated drinking water has been reported to be a risk factor for sporadic *Y. enterocolitica* infection in Norway (Ostroff *et al.*, 1994; Saebo *et al.*, 1994). Despite this drinking water has only been identified as the source of infection in a few episodes (Cover and Aber, 1989; Schiemann, 1990). In two outbreaks which involved small numbers of individuals, serotype 0:3 has been isolated from drinking water (Christensen 1979; Thompson and Gravel, 1986). The *Y. enterocolitica* 0:3 has been reported to grow in sterile spring water at 9°C (Karapinar and Gonui, 1991). Abraham *et al.* (1997) reported an outbreak of *Y. enterocolitica* from India in Tamil Nadu where by mistake the drinking water from a well contaminated by the bacterium was added to the butter milk. *Y. enterocolitica* has also been isolated from sewage water in New Delhi and the strains isolated were capable of survival and proliferation in mixed cultures, in sterile water at 4°C, 25°C and 37°C (Highsmith *et al.*, 1977).

*Y. enterocolitica* has been isolated from milk and milk products, egg products, raw meat and poultry, vegetable and many other food products Wauters (1991) has indicated that *Y. enterocolitica* isolated from food are mostly not pathogenic. Pasteurized Milk (de Boer *et al.*, 1986; Walker and Gilmour, 1986; Christensen, 1987b; Hughes, 1987; Rea *et al.*, 1992) and raw milk (Hughes 1987; Walker and Gilmour, 1986) having strains of *Y. enterocolitica* have been described. Walker and Gilmour, 1986 reported about 10-18% incidence of different strains of *Y. enterocolitica* in raw goat milk and these isolates have been implicated in human illness in many countries. There have been a few reports of isolation on *Y. enterocolitica* from pasteurized milk (Walker and Gilmour, 1986). *Y. enterocolitica* was isolated from 9.2% cheese and curd samples but not from cheddar cheese (Schiemann, 1987). In addition, six major outbreaks were associated with chocolate milk (Black *et al.*, 1978), Powder milk and chowmein (Shayegani *et al.*, 1983), Tofu (Tacket *et al.*, 1985), bean sprouts (Cover and
Aber, 1989) and pasteurized milk (Akers et al., 2000). A rarer serotype 0:13, has caused an outbreak where pasteurized milk was the common source (Tacket et al., 1984). Brocklehurst and Leistner (1975) reported that 28.9% of chicken samples, 34.5% of pork samples, and 10.6% of beef samples obtained from the retail market were contaminated with *Y. enterocolitica*. Several strains of *Y. enterocolitica* were isolated from vaccum packed beef and lamb cuts (Hanna et al., 1979). Hanna et al. (1997) reported large increase in counts of *Y. enterocolitica* on experimentally inoculated raw or cooked beef or pork stored at 7°C for 10 days at 25°C for 24 hours but could not survive in beef roasts cooked to a final internal temperature of 60-62°C. The rates of isolation and contamination were more in the case of pork than other meat and meat products. Many other workers reported isolation of *Y. enterocolitica* from meat and meat products from different countries (Schiemann, 1980; Doyle and Hugdahl et al., 1983; Harmon, 1983; Fukushima, 1985).

Most of the *Y. enterocolitica* isolates recovered from environmental samples, including fodder, soil foliage, surface water, sewerage water and sludge, were non-pathogenic (Berzero et al., 1991; Cork et al., 1995; Fransen et al., 1996; Sandery et al., 1996; Waage et al., 1999).

**Pathogenesis of Yersinia enterocolitica**

*Yersinia enterocolitica* is an enteric pathogen associated with a wide spectrum of clinical and post immunological manifestations including acute gastroenteritis, mesenteric adenitis, septicemia, arthritis and erythema nodosum, predominantly affecting young children (Bottone and Robin, 1979). The bacterium was first isolated in Asia from Japan (Zen-Yoji and Maruyamma, 1972). Sporadic cases of human gastroenteritis due to *Yersinia enterocolitica* have been recognized with increasing frequency since then. The bacteria rivals *Salmonella* and even surpasses *Shigella* in causing acute enteritis. In several tropical countries such as Iran, Israel, South Africa and Brazil, *Yersinia enterocolitica* has been observed to be a significant cause of human infections. Similar to the other members of the genus *Yersinia*, the most common
presentation of infection with *Y. enterocolitica* is an acute, self-limited gastroenteritis, occurring in approximately half to two thirds of all cases. It is characterized by diarrhea, abdominal pain and fever. The intensity and duration of diarrhea are variable, often of short duration but frequently persisting for one to two weeks and occasionally up to one year (Ostroff *et al.*, 1992). Bloody diarrhea has been recognized only in patients under 18 years of age (Ostroff *et al.*, 1992). Excretion of the organism in the stools is reported to occur long after diarrhea subsides and is believed to contribute to spread illness within family and the community (Marks *et al.*, 1980).

*Y. enterocolitica* is an invasive organism that appears to cause disease by tissue destruction (O’Loughlin *et al.*, 1990). Researchers have elucidated several potential pathogenic properties, including chromosomally mediated effects (eg, attachment to tissue culture, production of enterotoxin) and plasmid-mediated mechanisms (eg, production of Vw antigens, calcium dependency for growth, autoagglutination). Invasion of human epithelial cells and penetration of the mucosa occurs in the ileum, followed by multiplication in Peyers patches. A 103-kd protein, known as invasin and determined by the *inv* gene, mediates bacterial invasion. (Young *et al.*, 1992; Heesemann *et al.*, 1983).

A pathogenicity island present only in highly pathogenic strains of *Yersinia* (*Yersinia enterocolitica* 1B, *Y. pseudotuberculosis* I and *Y. pestis*) has been identified on the chromosome of *Yersinia* spp. and has been designated High-Pathogenicity Island (HPI). The *Yersinia* HPI carries a cluster of genes involved in the biosynthesis, transport and regulation of the siderophore yersinia bactin. The major function of this island is thus to acquire iron molecules essential for *in vivo* bacterial growth and dissemination. The presence of an integrase gene and sites homologous to those of phage P4, together with a G + C content much higher than the chromosomal background, suggests that the HPI is of foreign origin and has been acquired by chromosomal integration of a phage. A unique characteristic of the HPI is its wide distribution in various entero bacteria. Although first identified in *Yersinia spp.*, it has subsequently been detected in other genera such as *E. coli, Klebsiella* and *Citrobacter* (Camiel, 1999; Camiel,
The high pathogenicity island of *Yersinia enterocolitica* is also proved to be responsible for causing disease in a mouse model (Schubert et al., 2004).

As a foodborne pathogen, *Yersinia enterocolitica* can efficiently colonize and induce disease in the small intestine. Following ingestion, the bacteria colonize the lumen and invade the epithelial lining of the small intestine, resulting in the colonization of the underlying lymphoid tissues known as Peyer patches. A direct lymphatic link between the Peyer's patches and mesenteric lymph nodes may result in bacterial dissemination to these sites, resulting in mesenteric lymphadenitis (Jansson et al., 1968). Dissemination to extra intestinal sites, such as the spleen, is hypothesized to occur via two main mechanisms: colonization of the Peyer's patches, which can then be used as a staging ground for spread into the blood and or lymph, ultimately resulting in the appearance of bacteria in other tissues and bypass of the Peyer's patches and straight to systemic colonization (Olinde et al., 1984). Furthermore, the possibilities of additional avenues for dissemination have yet to be elucidated.

*Yersinia enterocolitica* colonization of the intestinal lymphoid tissues requires transmigration of the bacteria from the intestinal lumen across an epithelial tissue barrier. Specifically, antigen-sampling intestinal epithelial cells known as M cells are thought to be critical for this transmigratory process. The epithelium overlying the Peyer's patches has a high concentration of M cells; however, these cells have recently been identified throughout the non–Peyer's patch areas of the small intestine (Handley et al., 2005).

Furthermore, *Yersinia enterocolitica* and the related pathogen *Yersinia pseudotuberculosis* produce at least 3 invasion proteins, invasin, *ail*, and YadA, which can potentially promote adherence to and invasion of M cells. Invasin, the principle invasion factor of *Yersinia enterocolitica* and *Y pseudotuberculosis*, binds to β1 integrin receptors with high affinity, promoting internalization. These receptors are found at high levels on the luminal side of M cells but not on the luminal side of enterocytes. (Handley et al., 2005).
Drainage into the mesenteric lymph nodes can lead to systemic infection or mesenteric adenitis. The enterotoxin produced by Yersinia enterocolitica is similar to the enterotoxin produced by the heat-stable Escherichia coli; however, it likely plays a minor role in causing disease, as diarrheal syndromes have been observed in the absence of enterotoxin production. In addition, the toxin does not appear to be produced at temperatures higher than 30°C. The plasmid-mediated outer membrane antigens are associated with bacterial resistance to opsonization and neutrophil phagocytosis (Portnoy and Martinez, 1985).

One unique property of Yersinia enterocolitica is its inability to chelate iron, which is an essential growth factor for most bacteria and is obtained through the production of chelators known as siderophores. Yersinia enterocolitica does not produce siderophores but can utilize siderophores produced by other bacteria (eg, desferrioxamine E produced by Streptomyces pilosus). Iron overload substantially increases the pathogenicity of Yersinia enterocolitica perhaps through attenuation of the bactericidal activity of the serum (Arpi et al., 1991). Researchers observe differences in the iron requirements between different serotypes of the organism. This may explain, in part, the varying degrees of virulence of certain serotypes.

After an incubation period of 4-7 days, infection may result in mucosal ulceration (usually in terminal ileum and rarely in ascending colon), necrotic lesions in Peyer’s patches, and mesenteric lymph node enlargement. In severe cases, bowel necrosis may occur as a result of mesenteric vessel thrombosis (Bradford et al., 1974). Focal abscesses may occur. In persons with HLA-B27, reactive arthritis is not uncommon, possibly because of the molecular similarity between HLA-B27 antigen and Yersinia antigens. (Aho et al., 1974). The pathogenesis of Yersinia associated erythema nodosum is unknown.

The invasive capacity of Yersinia enterocolitica is associated with possession of a (40-48) x 10^6-mol. wt plasmid that provides the bacteria with various novel characteristics, including increased virulence for laboratory animals, a dependence on calcium for growth, enhanced binding of haemin and
Congo red, and altered surface properties that cause autoagglutination, resistance to the bactericidal effects of human serum, expression of new outer-membrane proteins and increased hydrophobicity (Portnoy et al., 1981; Martinez, 1983; Prpic et al., 1983).

In addition to their enteroinvasive property, many isolates of *Yersinia enterocolitica* from food or clinical material also produce a heat-stable enterotoxin, whose physicochemical properties, antigenicity and mechanism of action are very similar to those of the heat stable enterotoxin of *E. coli* (Robins-Browne et al., 1979; Okamoto et al., 1983). Genes for enterotoxin production are probably borne on the bacterial chromosome, and, in contrast to the plasmid-encoded characteristics, are typically expressed during growth at temperatures between 20 and 30°C (Robins-Browne *et al.*, 1985). Some isolates of *Yersinia enterocolitica* exhibit phospholipase activity, which has been linked to lecithin-dependent hemolysis (Tsubokura *et al.*, 1979). Connexin (Cx) channels are sites of cytoplasmic communication between contacting cells. Evidence indicates that the opening of hemichannels occurs under both physiological and pathological conditions. The involvement of Cx-43 hemichannels is demonstrated in the pathogenesis of *Yersinia* (Velasquez Almonacid *et al.*, 2009). All *Yersinia* spp. pathogenic to humans (*Y. pestis, Yersinia enterocolitica* and *Y. pseudotuberculosis*) harbor a highly conserved 70-kb plasmid (pYV) that is essential for virulence. This plasmid contains 50 virulence genes encoding an elaborate type III secretion system (ysc) and several proteins called Yops that are secreted upon contact with eukaryotic host cells. Some of the Yops (including YopE, YopH, YopO, and YopM) are delivered into the host cell cytosol where they damage the cytoskeleton and disrupt the signaling network (Rosqvist *et al.*, 1994; Sory and Cornelis, 1994; Sory *et al.*, 1995; Håkansson *et al.*, 1996; Boland *et al.*, 1996). These Yop effectors are translocated across the eukaryotic cell membrane by a specialized apparatus made up of several other Yops including YopB and YopD (Håkansson *et al.*, 1996). Secretion of Yop effectors and translocators requires specific bacterial chaperones, called Syc proteins (Wattiau *et al.*, 1996). Related type III secretion systems have been encountered in
various other animal pathogenic bacteria such as *Pseudomonas aeruginosa* (Exs/Psc), enteropathogenic *Escherichia coli* (Sep), *Shigella* spp. (Mxi/Spa), and *Salmonella* spp. (Inv/Spa), as well as plant pathogenic bacteria such as *Xanthomonas campestris* (Hrp), *Pseudomonas syringae* (Hrp), and *Erwinia* spp. (Hrp) (Finlay and Cossart, 1997; Lee, 1997). For visible pathology to develop, a threshold number of bacteria (>10^5) is needed and the bacteria need to persist for more than 24 hrs (Pepe et al., 1995).

Histologic findings in *Yersinia enterocolitica* infection are consistent with acute and chronic inflammation. Yersiniosis does not produce unique histologic findings. Epithelial cell granulomas with suppurative centers of the granulomas (central microabscesses) have been reported. These granulomas are composed of numerous histiocytes with or without epithelioid cell features, along with scattered small T-lymphocytes and plasmacytoid monocytes (Kojima et al., 2007). It is demonstrated by scanning electron microscopy that the follicle associated epithelium is the primary site of host-pathogen interaction in *Yersinia enterocolitica* infection and that this pathogen penetrates M cells of intestine and subsequently induces destruction of the Payer’s patches (Autenrieth and Firsching, 1996). Enteropathogenic *Yersinia* species express the afimbrial adhesin YadA, the prototype of a class of homo trimeric outer membrane adhesins, which mediates adherence to host cells by binding to extracellular matrix components (Heise and Dersch, 2006). The host cytokines IFN-gamma and TNF-alpha as well as the integrins Mac-1 and VLA-4 are involved in protective cellular host defense mechanisms in Payer’s Patches and mesenteric lymph nodes against *Yersinia enterocolitica*, the latter probably being involved in both cell-cell and cell-pathogen interactions (Autenrieth et al., 1996). *Yersinia enterocolitica* invasin (Inv) protein confers internalization into and expression of proinflammatory cytokines by host cells. Both events require binding of Inv to β1 integrins, which initiates signaling cascades including activation of focal adhesion complexes, Rac1, mitogen-activated protein kinase, and NF-κB (Bühler et al., 2006). YadA, in addition to Inv, YopB, and *Yersinia* lipopolysaccharide, is a further inducer of proinflammatory molecules by which *Yersinia enterocolitica*
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might promote inflammatory tissue reactions (Schmid et al., 2004). *Yersinia enterocolitica* isolates of differing biotypes from humans and animals are adherent, invasive and persist in macrophages, but differ in cytokine secretion profiles *in vitro* (McNally et al., 2006). IFN-γ-mediated effector mechanisms can partially compensate virulence exerted by YopH of *Yersinia enterocolitica* (Matteoli et al., 2008).

Patients who are homozygous for thalassemia major are at risk for *Yersinia enterocolitica* infections (Hansen et al., 2001). *Yersinia enterocolitica* is a possible pathogen in febrile chronically transfused patients with sickle cell disease (Blei and Puder, 1993). Infections due to *Yersinia enterocolitica* are usually limited to the bowel. When infection is generalized, the role of iron overload is widely acknowledged (Paitel et al., 1995; Boelaert et al., 1987). There is also a considerable infection risk of the pathogen in haemodialysis patients and transplant recipients (Hoen and Kessler, 1991). In the context of iron overload and haemodialysis *Yersinia enterocolitica* septicemia is also described (Paitel et al., 1995; Hoen et al., 1988). In the patients suffering from haemochromatosis the pathogen causes multiple hepatosplenic abscesses in addition to gastroenteritis (Zapata and Garcia; 1997; Bergmann et al., 2001; Abdelli et al., 1996). *Yersinia enterocolitica* infection with ileal perforation associated with iron overload and deferoxamine therapy has been described (Mazzoleni et al., 1991) *Yersinia enterocolitica* septicemia with autologous blood transfusion is known (Haditsch et al., 1994; Benavides et al., 2003). *Yersinia enterocolitica* contaminated blood is a rare cause of potentially fatal post transfusion septicemia (Beresford, 1995). Apart from gastrointestinal illness *Yersinia enterocolitica* infection is thought to be a causative factor for urticaria (Hellmig et al., 2009).

**Molecular pathogenesis**

*Yersinia enterocolitica* has evolved into an apparently heterogeneous collection of organisms encompassing six biotypes differentiated by physiochemical and biochemical tests (1A, 1B, 2, 3, 4, and 5) and more than 50...
serotypes differentiated by antigenic variation in cell wall lipopolysaccharide. Of the six biotypes, biotype 1A is the most heterogeneous, and encompasses a wide range of serotypes, of which serotypes O:5, O:6,30, O:6,31, O:7,8, O:10, as well as O-nontypable strains, are isolated most often (Tennant et al., 2003.) The virulence of the pathogenic biotypes, namely, 1B and 2–5 is attributed to the presence of a highly conserved 70-kb virulence plasmid, termed pYV/pCD and certain chromosomal genes (Cornelis et al., 1998). The biotype 1A strains of Y. enterocolitica, on the other hand, have been reported to lack pYV plasmid which encodes virulence factors including Yersinia adhesin A (YadA) and Ysc-Yop type III secretion system (TTSS) as well as chromosomally borne virulence genes including ail, myfA, ystA, ysa, and the high pathogenicity island- (HPI-) associated iron acquisition system (Bhagat and Virdi, 2007). Apart from pYV itself, pYV-bearing strains of Y. enterocolitica require a number of chromosomally borne genes to express full virulence. Some of these virulence genes are restricted to pYV-bearing bacteria whereas others occur more widely. Virulence genes that are mostly limited to pYV-bearing strains of Y. enterocolitica include inv (encodes invasin, an outer membrane protein that is required for efficient translocation of bacteria across the intestinal epithelium (Pepe and Miller, 1993) ail (encodes another outer membrane protein that may contribute to adhesion, invasion, and resistance to complement-mediated lysis) (Miller et al., 1988) yst encodes Yersinia heat-stable enterotoxin that may contribute to the pathogenesis of diarrhea associated with acute yersiniosis (Delor and Cornelis, 1992); myf (encodes a fimbrial antigen and putative adhesion (Iriarte et al., 1993). In addition, strains of biotype 1B, which are particularly virulent for humans and laboratory animals, carry a high-pathogenicity island (HPI) which facilitates the uptake and utilization of iron by bacterial cells, and hence may promote their growth under iron-limiting conditions in host tissues (Carniel, 1999.) Virulence-associated determinants of pYV-bearing Y. enterocolitica that also occur in pYV-negative strains include cell surface lipopolysaccharide and SodA (a superoxide dismutase), which appear to facilitate bacterial survival in tissues (Roggenkamp et al., 1997; Zhang et al., 1997.), as well as urease, which enhances bacterial
resistance to stomach acid and may also play a role in nitrogen assimilation (De Koning-Ward and Robins-Browne, 1995). pYV functions mainly as an antihost plasmid that permits the bacteria which carry it to resist to phagocytosis and complement-mediated lysis, thus allowing them to proliferate extracellularly in tissues. The pYV plasmid encoded virulence factors include an outer membrane protein adhesin, YadA, and a type III protein secretory apparatus which translocates effector proteins, known as Ysc-Yops, from the bacterial cell to the cytoplasm of susceptible host cells (Cornelis et al., 1998). The contribution of pYV-encoded factors, in particular YadA and the Yop effectors, to bacterial virulence has been established in a large number of studies. Strains of *Yersinia* which lack pYV are susceptible to killing by complement and polymorphonuclear leukocytes, although they are able to persist in macrophages and nonprofessional phagocytic cells, and cause short-lived infections which are typically asymptomatic.

Biotype 1A strains of *Y. enterocolitica* are often considered to be nonpathogenic primarily because they do not possess the virulence-associated factors of pYV-bearing strains. The biotype 1A strains have been reported to lack both pYV plasmid and most chromosomal virulence genes such as *ail*, *myfA*, *ystA*, *ysa*, TTSS, and HPI, and only occasionally carry *ystA* and *myfA* (Kot et al., 2010). Although the *ail* gene is present in some biotype 1A strains, the *ail* gene alone is an insufficient virulence marker for detecting the virulence of *Y. enterocolitica* biotype 1A strains (Sihvonen et al., 2011). Another line of evidence that is taken to indicate the avirulence of biotype 1A strains is their relatively high prevalence in the environment and healthy animals. Indeed, biotype 1A strains are ubiquitous, inhabiting a wide variety of environmental niches such as soil and various sources of water, including streams, lakes, water wells, and wastewater (Shayegani et al., 1986). They are also frequently isolated from foods, including various vegetables and animal products, such as pork, poultry, packaged meat, seafood, raw milk, and pasteurized dairy products. BiotypelA are also found in a vast array of animals, including birds, fishes, various insects, frogs, and a wide range of mammals, including cattle, sheep,
pigs, and rodents. In most cases, animals infected with biotype 1A strains are asymptomatic, thus giving support to the concept that these bacteria are avirulent commensals (Tennant et al., 2003). Despite the lack of traditional chromosomal-borne and plasmid-borne virulence genes in *Y. enterocolitica* strains of biotype 1A, some biotype 1A strains are isolated frequently from humans with gastrointestinal diseases. The biotype 1A strains isolated from humans and from pigs have been reported to produce *ystB*-encoding *Yersinia* heat-stable enterotoxin (Kot et al., 2010). A recent study on 259 isolates of *Y. enterocolitica* and related species; indicated that Yst B (*ystB*) was the major contributor to diarrhea produced by biotype 1A strains of *Y. enterocolitica* (Singh and Virdi, 2004). Some biotype 1A strains produce symptoms indistinguishable from that produced by isolates belonging to pathogenic biotypes (Morris et al., 1976.). Biotype 1A strains have also been implicated in nosocomial (Ratnam et al., 1982) and foodborne (Greenwood and Hooper, 1990) outbreaks, and were also isolated from extraintestinal infections (Bissett et al., 1990).

*Yersinia enterocolitica* pathogenesis is incompletely understood. Most isolates of *Y. enterocolitica* from food or clinical materials have either of two pathogenic properties. First property is the ability to penetrate the intestinal wall, which is thought to be controlled by 70-kb virulence plasmid (pYV/pCD) genes; that is absent in avirulent strains; second one is the production of heat-stable enterotoxin which is controlled by chromosomal genes (*ystA*, *ystB*, and *ystC*) (Robins-Browne et al., 1985). Adaptation, as contaminated foods are considered as the common mode of transmission, this microorganism must first adapt its surface antigenic structures like outer membrane proteins to colonize in the intestines of humans at a temperature of about 37 °C. This is usually achieved in part through the presence of 70-kb virulence plasmid (pYV). Genes on this plasmid encode for several outer membrane proteins (polypeptides) that are expressed at 37 °C but not at 25 °C (Bottone, 1997).

Adhesion attachment of pYV-bearing strains (pathogenic biotypes 1B and 2–5) of *Y. enterocolitica* to tissue culture cells like HeLa cells or HEp-2 cells culture has been frequently identified in pathogenic *Yersinia* isolates (Mors and
Pai, 1980; Vesikari et al., 1981). However, the ability to produce disease does not correlate with HeLa cell attachment as plasmid cured avirulent strains retain the ability to attach to HeLa cells (Schiemann and Devenish, 1982.). When the pYV plasmid-containing strain was grown at 26 °C in calcium-containing medium, the bacteria adhered to HeLa cells and HEp-2 cell cultures to a high degree. In contrast, when this strain was incubated at 37 °C in the same calcium-containing medium, it attached to the HeLa cells and HEp-2 cell cultures at a reduced level (Bolin et al., 1988). By insertional inactivation of genes located on the virulence plasmid (pYV), Kapperud et al. (1987), identified four plasmid dependent and temperature-inducible properties related to the bacterial surface properties involved in fimbrial adhesion: (i) a fimbrial matrix covering the outer membrane, (ii) outer membrane protein, YOP1 which is a structural component of the fimbriae, (iii) spontaneous autoagglutination, which is related to the fimbriae, and (iv) mannose-resistant hemagglutination of guinea pig erythrocytes (Kapperud et al., 1987). Although the biotype 1A strains of Y. enterocolitica have been reported to lack pYV plasmid, various forms of fimbriae are observed in this biotype. One of fimbriae, designated MR/Y-HA is 8 nm in diameter, agglutinates erythrocytes of 10 different animal species in the presence of mannose and is expressed in vitro at low temperature, but not at 37 °C (Old and Adegbola, 1984). A second type of fimbriae, designated MR/Klike HA is 4 nm in diameter and mediates mannose resistant hemagglutination of chicken erythrocytes, but not erythrocytes from a variety of other species (Old and Adegbola, 1984). Expression of these fimbriae in vitro occurs only after serial passages of bacteria for at least 7 days. Moreover, as they do not mediate adherence of bacteria to cultured epithelial cells (Old and Robertson, 1981), their contribution to the pathogenesis of infection with biotype 1A strains is unknown (Tennant et al., 2003.) Some strains of Y. enterocolitica produce a fimbrial adhesin, named Myf (for mucoid Yersinia fibrillae), because it bestows a mucoid appearance on bacterial colonies which express it. Myf are narrow flexible fimbriae which resemble CS3, an essential colonization factor of some human clinical strains of enterotoxigenic Escherichia coli (Tennant et al., 2003). However, myf genes-
associated virulence of these bacteria is unknown. The ability of *Y. enterocolitica* to invade epithelial cells is an important correlation of pathogenicity (Miller *et al.*, 1988). The invasive process includes a major signalling process that an invasive microorganism may provoke to force its way into a nonphagocytic cell, and then disrupting and invading the intestinal barrier, a process that involves interaction with other cellular components of this barrier. There are essentially two major mechanisms of bacterial epithelial cell internalization (Isberg, 1991). The “zippering” process corresponds to tight enclosing of the bacterial cell by the mammalian cell membrane, involving a surface bound bacterial protein binding an adherence molecule of the mammalian cell surface with high affinity—that is, the invasin (Inv) of *Yersinia* binding integrins of the β1 family of mammalian cell surface (Isberg and Barnes, 2001). One reason that strains of biotype 1A have been considered to be avirulent is that they invade tissue culture cells to a lesser extent than pYV-bearing strains. However, paradoxically, some pYV-bearing strains themselves may retard mammalian epithelial cell invasion via the effects of translocated Yops on cytoskeletal proteins (Cornelis *et al.*, 1998), as well as some biotype 1A strains positive for the *ail* gene encoded an outer membrane protein that may contribute to epithelial cell adhesion and invasion (Miller *et al.*, 1990).

*Y. enterocolitica* usually causes a diarrheal disease, and sometimes systemic diffusion. *Yersinia* virulent strains cross the intestinal epithelium primarily through the FAE (follicle associated epithelial cell), in the Peyer’s patches of the ileum (Grutzkau *et al.*, 1990). Invasin (Inv), a 103 kDa outer membrane protein of *Yersinia* binds β1 integrins that are also expressed apically on M cells. Inv negative mutants still adhere to and invade M cells, but at a much lower level than the wildtype strain and their colonization potential for Peyer’s patches is considerably reduced (Clark *et al.*, 1998). Other *Yersinia* surface proteins such as Ail, PsaA, and YadA may account for residual invasion of inv mutants. (Marra and Isberg, 1999). After invasion process, *Yersinia* defend the attack by resident macrophages by expressing an antiphagocytic strategy mediated by a plasmid encoded type III secretion, of three protein effectors, YopH, T, and E, that disrupt
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cytoskeletal assembly required for phagocytosis process *Yersinia* strains therefore remain extracellular in infected Peyer’s patches and mesenteric lymph nodes, and then disseminate to cause local and systemic infection. (Fallman et al., 1997; Cornelis, 1998).

**Continental distribution and Prevalence**

*Y. enterocolitica* appears to be widely distributed in the environment. The highest incident of human infection due to *Y. enterocolitica* has been reported in Belgium and majority of the isolates belonged to serotype 0:3 and 0:9. The isolation of *Y. enterocolitica* was reported from France, Czechoslovakia and West Germany by different workers and they also reported that the majority of the isolates belonged to serotype 0:3 and 0:9 (Rakovsky et al., 1973). Human isolates of *Y. enterocolitica* were reported from Canada and USA by different investigators and serotype 0:8 was the most predominant among these isolates (Toma, 1973; Weaver et al., 1973). Zen-yoji and Maruyam (1972) reported on the first isolation of *Y. enterocolitica* from Japan. The majority of the yersiniae belonged to the serotype 0:3 and 0:6 0:3. The serotype 0:3 was the most predominant among the isolates in Israel. *Y. enterocolitica* 0:3 was identified as the causative agents of children infection in Brazil.

**Epidemiology of *Y. enterocolitica***

During the past 10 years, increasing evidence has accumulated that *Yersinia enterocolitica* infections are very frequent in some parts of the world, and the infection is probably common but unrecognized in many other countries. Only 23 cases of *Yersinia enterocolitica* infection were recorded in 1966, the number raised to 642 cases in 1970, approximately 1000 cases in 1972 and over 4000 in 1974 (Morris and Feeley, 1976). The increase in reported isolations is probably a result of greater awareness about this organism and about its potential role in human and animal disease. The first isolate of *Yersinia enterocolitica* was reported from Czechoslovakia in 1963 (Rakovsky et al., 1973). *Yersinia enterocolitica* infections are more frequent in children (commonly 3-5 years) than in adults (Esseveld and Goudzwaard, 1973). Although human
infections with \textit{Yersinia enterocolitica} have now been observed in numerous countries in all parts of the world, knowledge about the geographical distribution of this organism is extremely fragmentary. For reasons that are unexplained, reported incidence rates and distributions of serotypes seem to vary widely, and often abruptly, between neighboring countries (Arvastson \textit{et al.}, 1971).

The rate of infection varies with different geographical locations. Even within a given country there are striking regional differences (Morris and Feeley, 1976). The pathogenicity of \textit{Yersinia enterocolitica} appears to depend on the method of cultivation and the site of inoculation (Rokovsky, 1973). In addition to human infection \textit{Yersinia enterocolitica} has also been isolated from animals including dogs (Wilson \textit{et al.}, 1976), pigs (Esseveld and Goudzwaard, 1973; Zen-Yoji and Sakai, 1974) and from food stuffs, such as ice-cream and oysters (Toma, 1973). The organism is commonly found in specimens from swine slaughter houses and has been isolated from samples of market meat and vacuum-packed beef (Fukushima, 1985). It has also been found in drinking water (Lassen, 1972) usually in non chlorinated well water (Saari and Quan, 1976; Highsmith \textit{et al.}, 1977). Very little is known about how \textit{Yersinia enterocolitica} spreads; however, in one outbreak in Japan (Askawa \textit{et al.}, 1973) a common source, possibly food, is implicated. A food source was also implicated in an outbreak in Czechoslovakia in which the food may have been contaminated by a food handler (Olsousky, 1975). It is generally felt that the disease probably has a fecal origin. Hospital outbreaks and family and interfamilial outbreaks suggest that transmission through personal contact may occur (Kellogg \textit{et al.}, 1995). Data for the number of \textit{Yersinia enterocolitica} organisms required to cause human disease are very limited (Szita \textit{et al.}, 1973).

Mass production of animals, development of meat factories based on sophisticated chains of cold storage units and international trade of meat products and animals are believed to be the reasons for the increasing prevalence of Yersiniosis in humans. In Germany, anti-\textit{Yersinia} antibodies were found in up to 40\% of the average population (Neubauer \textit{et al.}, 2001). High frequency of anti-Yersinial antibody was also observed from sera of healthy
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Finland (Mäki-Ikola et al., 1997) and Italy subjects (Franzin and Curti, 1993). Human race outbreaks of *Yersinia enterocolitica* induced acute diarrhea are now common. An outbreak involving 11 persons infected with *Yersinia enterocolitica* O:9 was reported from Norway in February 2006. Outbreak of *Yersinia enterocolitica* O:8 infections associated with pasteurized milk was also described from the upper valley of Vermont and New Hampshire (Ackers et al., 2000). A multi state outbreak of infections caused by *Yersinia enterocolitica* transmitted by pasteurized milk from states of Tennessee, Arkansas, and Mississippi was recorded (Tacket et al., 1984). Outbreak of food poisoning induced by *Yersinia enterocolitica* has also been reported from Japan (Sakai et al., 2005). An outbreak of acute diarrhea episodes caused by the pathogen was also reported from Croatia (Babić-Erceg et al., 2003) and New York (Shayegani et al., 1983). Isolation of *Yersinia spp.* from cases of diarrhoea in infants and children has recently been reported from Iraq (Kanan and Abdulla, 2009; Soltan-Dallal and Moezardalan, 2004). A bacteriologically confirmed case of *Yersinia enterocolitica* was reported from Poland since 2003. In the first epidemiological report on Yersiniosis in Poland in 2006, 140 confirmed case of *Yersinia enterocolitica* were observed. Out of them, 39 cases belonged to serotype O3 of *Yersinia enterocolitica* and the most important finding was the occurrence of cases caused by serotype O8 (Bobel and Sadkowska-Todys, 2008). In 2007, 233 Yersiniosis cases were reported from Poland out of which 182 cases were of enteric Yersiniosis and 51 cases were of extra intestinal Yersiniosis. Most of them were serotype O3 but 11 cases were caused by serotype O8 (Napiórkowska et al., 2009). In Finland *Yersinia enterocolitica* induced gastroenteritis is reported since 1995 with the number of cases ranging between 12.4-17.9 per 100,000 population during 1995-2001 (Korte et al., 2004). *Yersinia enterocolitica* has been isolated from sources of drinking water in Izmir city (Turkey) which is believed to be a potential cause of gastrointestinal outbreaks (Göñül and Karapınar, 1991). Young children account for a large proportion of reported *Yersinia enterocolitica* infections in Sweden with a high incidence compared with other gastrointestinal infections, such as Salmonellosis and
Campylobacteriosis (Boqvist et al., 2009). *Yersinia enterocolitica* was reported to be a common cause of gastroenteritis among others in Southern Germany during 2000-2006 (Bucher et al., 2008).

In spite of much concern about *Yersinia enterocolitica* induced gastroenteritis in many parts of the globe and some reports of *Yersinia enterocolitica* associated diarrhea outbreaks in India, the highly pathogenic 1B *Yersinia enterocolitica* has not reported from India so far. Therefore intense search for the highly pathogenic *Yersinia enterocolitica* strain from any part of India is justified and should be of considerable recent interest. In light of this, the present study was carried out with following aims and objectives:

**Aims and Objectives**

The overall aim of the work was to isolate and characterize *Yersinia enterocolitica* from stool samples of diarrheal patients in and around Chandigarh. To fulfill the above mentioned aim, the study was planned with the following objectives:

1. Isolation of *Yersinia enterocolitica* in and around Chandigarh from variable sources.
2. To characterize the isolated strains by biochemical method, serotyping and OMP’s profile.
3. Genetic characterization of isolated strains of *Yersinia enterocolitica* and comparison with known strain.
4. To detect and characterize the virulence pathogenicity of *Yersinia enterocolitica* on intestinal epithelium.
5. Histopathology of *Yersinia enterocolitica* infected intestine.